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Length and activation dependent variations in muscle shear wave speed

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Abstract

Muscle stiffness is known to vary as a result of a variety of disease states, yet current clinical methods for quantifying muscle stiffness have limitations including cost and availability. We investigated the capability of shear wave elastography (SWE) to measure variations in gastrocnemius shear wave speed induced via active contraction and passive stretch. Ten healthy young adults were tested. Shear wave speeds were measured using a SWE transducer positioned over the medial gastrocnemius at ankle angles ranging from maximum dorsiflexion to maximum plantarflexion. Shear wave speeds were also measured during voluntary plantarflexor contractions at a fixed ankle angle. Average shear wave speed increased significantly from 2.6 to 5.6 m s^{-1} with passive dorsiflexion and the knee in an extended posture, but did not vary with dorsiflexion when the gastrocnemius was shortened in a flexed knee posture. During active contractions, shear wave speed monotonically varied with the net ankle moment generated, reaching 8.3 m s^{-1} in the maximally contracted condition. There was a linear correlation between shear wave speed and net ankle moment in both the active and passive conditions; however, the slope of this linear relationship was significantly steeper for the data collected during passive loading conditions. The results show that SWE is a promising approach for quantitatively assessing changes in mechanical muscle loading. However, the differential effect of active and passive loading on shear wave speed makes it important to carefully consider the relevant loading conditions in which to use SWE to characterize *in vivo* muscle properties.

Keywords: shear wave elastography, muscle stiffness, gastrocnemius

(Some figures may appear in colour only in the online journal)

1. Introduction

Muscle mechanical properties are closely linked with neuromuscular health and disease. Abnormal muscle stiffness, for example, has been shown to be indicative of a variety of disease states including spasticity (Barrett 2011), Duchenne muscular dystrophy (Cornu *et al* 1997, 2001) and hyperthyroidism (Bensamoun *et al* 2007). However, it remains challenging to quantitatively assess *in vivo* muscle stiffness. Magnetic resonance (MR) elastography can be used to evaluate muscle stiffness from induced wave patterns, but is limited by the high imaging costs and availability of MR scanners. Ultrasonic shear wave elastography (SWE) is a new alternative approach for assessing spatial variations in tissue stiffness based on shear wave propagation speeds. Ultrasound SWE uses acoustic radiation force to induce transient shear waves within soft tissues, with wave speed then tracked using ultra-high frame rate ultrasonic imaging (Bercoff *et al* 2004). Shear wave speed is related to the shear modulus of elasticity of the tissue and hence is considered to be a non-invasive, quantitative metric of tissue stiffness (D'Onofrio *et al* 2010). Furthermore, ultrasonic motion tracking is sufficiently fast that it can be implemented on the scanner to provide real-time quantitative metrics of tissue stiffness.

Although SWE has proven successful in detecting pathological changes in stiffness in a variety of soft tissues (Sebag *et al* 2010, Bavu *et al* 2011, Athanasiou *et al* 2010), its application to skeletal muscle tissue is complicated by the fibrous structure of muscle, and the capacity for muscle tissue stiffness to vary with muscle length and activation level. An early study by Levinson *et al* (1995) established the use of ultrasound in measuring wave propagation induced by external vibrators through skeletal muscle and demonstrated that wave speed increased with applied load. Gennisson *et al* then applied this methodology to measure the anisotropic shear moduli of muscle in both *in vitro* and *in vivo* test cases (Gennisson *et al* 2003). More recent studies have provided further evidence of the capability of SWE to characterize skeletal muscle properties. Most notably, prior studies have shown that muscle shear wave speed increases proportionally with muscle activity (Nordez and Hug 2010, Gennisson *et al* 2005, 2010), the anisotropy of muscle tissue can be characterized with SWE (Lee *et al* 2012, Gennisson *et al* 2010), and that ultrafast ultrasound imaging can be used to characterize muscle contractions, both in terms of transient properties (e.g. contraction time) as well as two and three dimensional spatial maps of contraction propagation (Deffieux *et al* 2006, 2008). In 2010, Shinohara *et al* (2010) demonstrated that *in vivo* shear wave speeds can be modulated by altering gross body positioning, which changes muscle length, and a few recent publications have demonstrated SWE to be capable of capturing the inherent strain-stiffening behaviour of passive muscle, by showing that shear wave speed increases in stretched muscle (Maisetti *et al* 2012, Nordez *et al* 2008, Gennisson *et al* 2010).

Since muscle stiffness is known to increase monotonically with load (Durfee and Palmer 1994), these previous observations suggest that SWE could potentially provide a tool by which to track functional variations in muscle force. Physiologically, net muscle force is generated from both muscle activation and intrinsic passive factors, such as the stretch of connective tissues within and surrounding the muscle. Thus, it is important to assess if shear wave speed responds similarly to force generated via active and passive processes. In the current study, we investigated the relative effect on shear wave speed of load induced via voluntary activation and postural manipulation. We hypothesized that shear wave speed would increase due to both muscle activation and passive stretch, and that in both cases the increase in shear wave speed would be proportional to the net load induced about the musculoskeletal joint.

2. Methods

Institutional Review Board approval and informed consent were obtained. Ten healthy young adults were recruited for this study (table 1). Subjects were asked to walk at a comfortable pace for 6 min to pre-condition the muscle-tendon unit (Hawkins *et al* 2009). All testing was then performed with the subject lying prone on an examination table. A twin-axis

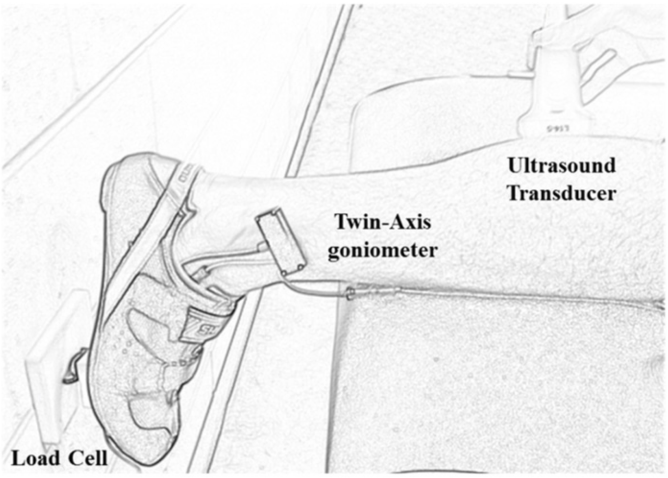

Figure 1. Experimental setup. Ultrasonic data were collected from the medial gastrocnemius muscle belly. Load information was collected from a load cell attached via a cycling shoe, and angle data were collected from a twin-axis electronic goniometer attached at the ankle joint. During active trials, the subject was instructed to push against the wall directly behind the foot as shown in the image. During passive trials, the foot was manually moved by a researcher to specific sagittal ankle angles.

Table 1. Subject information. Please note that positive angles denote plantarflexion.

			MVC (Nm)	Range of Motion (°)			
			Knee extended	Knee extended		Knee flexed	
Subject	Age	M/F		Max. dorsiflexion	Max. plantarflexion	Max. dorsiflexion	Max. plantarflexion
1	22	F	34	−8	42	−29	47
2	21	M	37	−12	39	−23	41
3	24	M	47	−12	39	−33	28
4	21	F	54	−8	42	−23	31
5	22	M	41	−11	32	−20	29
6	26	F	41	−23	32	−28	30
7	21	M	59	−12	30	−24	29
8	38	M	40	−7	45	−6	50
9	31	M	52	−14	40	−24	38
10	26	M	44	−15	29	−24	38

electronic goniometer (SG110/A, Biometrics Ltd, Newport, UK) was attached across the ankle to monitor sagittal ankle angle throughout testing. A load cell (LLB400, Futek Advanced Sensor Technology, Irvine, CA) was attached under the forefoot of a rigid-soled cycling shoe to measure the foot force (figure 1).

Imaging was performed using a linear array ultrasound transducer (L15–4, Supersonic Imagine, Aix-en-Provence, France) placed over the medial gastrocnemius muscle belly of the right limb. The transducer was centred 5 cm proximal to the distal muscle-tendon junction of the gastrocnemius. Care was taken during collections to move the probe such that data were collected from a similar location within the muscle belly for all collections. Ultrasonic B-mode images and shear wave data were collected using an Aixplorer clinical scanner

(Supersonic Imagine, Aix-en-Provence, France), with software version 5. The musculoskeletal preset was used for collections with the persist and smoothing parameters set to high and 7 respectively.

Ultrasound and shear wave data were collected while the ankle was statically held at ankle angles ranging from maximum dorsiflexion to maximum plantarflexion. The range of motion of each subject was established by passively moving the subjects' ankle through its range of motion first with the subjects' knee extended (0° knee flexion) and then with the knee flexed (90°). The flexed knee posture shortens the gastrocnemius considerably, such that the net force under the foot is primarily due to stretch of the soleus under that condition. Passive load data were measured when the ankle was moved through its range of motion so that the passive moment could be compared to the moments generated from the maximum voluntary contraction (MVC) of each subject. Shear wave images were collected with the subjects' ankle held in place by a researcher at set ankle angles ranging from maximum dorsiflexion to maximum plantarflexion, with 15° increments between postures. Specific angles (0°, 15°, 30°) were collected from all subjects, with –15° and 45° added if these fell within the subjects' range of motion. Data were collected in a random order with three trials of five images collected for each posture, leading to between 33 and 42 passive trials for each subject. For the flexed knee trials, subjects were instructed to leave their lower limb resting on the mat and to support themselves by bending at the knees and the waist.

Gastrocnemius shear wave speed, foot force and ankle angle data were also collected from a series of trials in which subjects were asked to actively generate ankle plantarflexor contractions. We first measured each subject's MVC by asking them to push as hard as they could against the wall located directly behind them. Subjects were then asked to sustain fixed contractions at six levels of effort (12.5%, 25%, 37.5%, 50%, 75%, and 100% of MVC) by again pushing against a fixed support. Ultrasound data were simultaneously collected from the medial gastrocnemius muscle belly, with three trials of five images collected for each activation level in a random order, for 18 total active trials per subject.

For each trial, shear wave speeds were averaged within a circular (∼10 mm diameter) region of interest (ROI) that was manually defined to lie within the medial gastrocnemius muscle belly (figure 2). The ROI was defined using a plug-in for the DICOM reader OsiriX (OsiriX Foundation, Geneva, Switzerland). This process was independently repeated by a second rater, and an intraclass correlation coefficient (ICC(2,1)) was used to assess inter-rater repeatability. We separately determined the average foot force and ankle angle for each trial using custom MATLAB (Mathworks, Natick, MA) routines. Foot force data were multiplied by the perpendicular distance between the load cell and ankle joint centre to ascertain the net internal ankle plantarflexion moment. Joint moments (M) were normalized to the moment achieved during the MVC (M_{MVC}) for each subject. Repeated measures analysis of variation (ANOVA) was used to evaluate the influence of activation level and posture on shear wave speeds. Any significant effects were followed up with post-hoc Tukey comparisons and a significance level of $p < 0.05$ was used for all tests. All results are reported mean (s.d.) across subjects unless otherwise noted.

3. Results

The relaxed gastrocnemius exhibited an average shear wave speed of 2.1 (0.3) m s^{-1}, as was observed with the knee flexed and the ankle in the most plantarflexed posture. Passive dorsiflexion of the ankle in a flexed knee posture did not induce a significant change in shear wave speed. However, passive dorsiflexion of the ankle with an extended knee resulted in the gastrocnemius muscle shear wave speed significantly increasing to 5.6 (1.5) m s^{-1} in

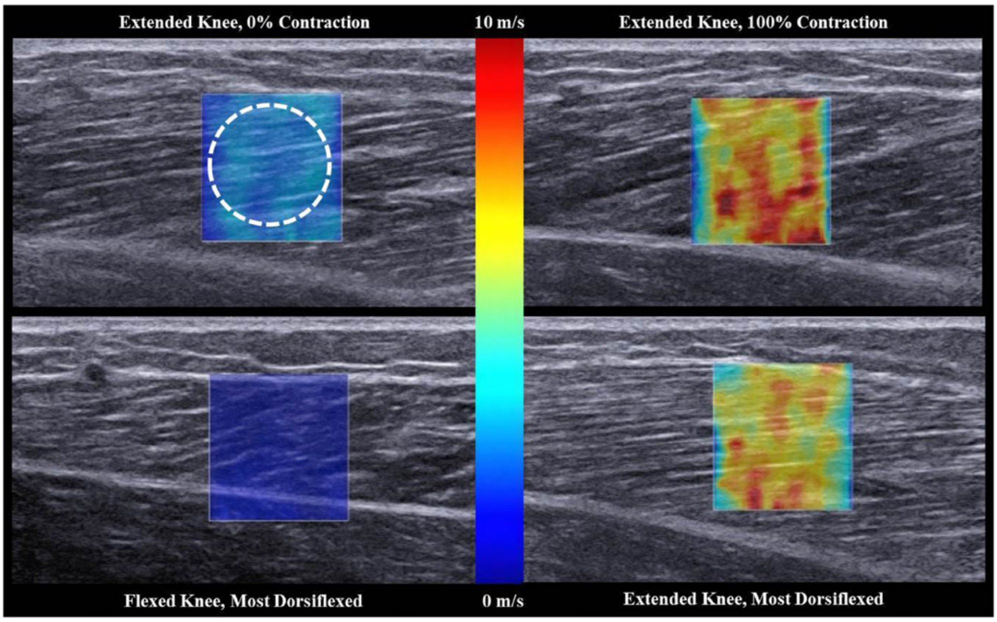

Figure 2. Data were collected in the medial gastrocnemius muscle belly. An increase in shear wave speed can be seen clearly when the subject is asked to apply a high load. When the ankle is dorsiflexed, the shear wave speed is noticeably higher in the extended knee posture. The data from subject 6 are shown. The image in the top left shows an example ROI defined within the gastrocnemius muscle belly.

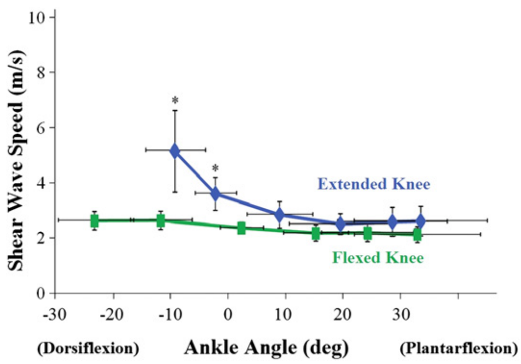

Figure 3. Passive ankle dorsiflexion induced a significant increase in shear wave speed when the knee was extended, but had little effect on shear wave speed when the knee was flexed. $^*p < 0.05$ in post-hoc comparisons.

the most dorsiflexed posture (figure 3). For individual subjects, shear wave speeds were linearly correlated with the passive net ankle moment, with correlation coefficients averaging 0.8 (0.2).

During active contractions, shear wave speed monotonically varied with the normalized ankle moment generated (M/M_{MVC}), increasing from 3.6 (0.6) m s^{-1} in the 0% effort condition to 8.3 (1.0) m s^{-1} in the maximally contracted condition (figure 4). The linear correlation

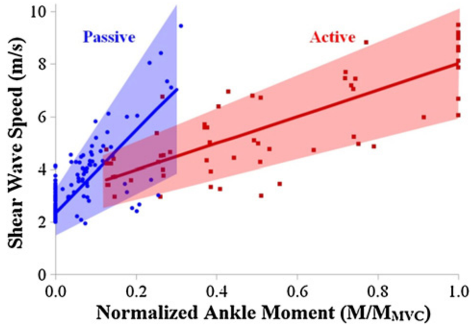

Figure 4. Shear wave speed was highly correlated to normalized ankle moment induced via passive stretch (extended knee) or active contraction. However, the slope of the best linear fit was significantly greater in the passive loading condition. The average (± 1 deviation) of trend lines across subjects is plotted, as well as data points from all trials.

Table 2. Active and passive trend lines for shear wave speed to normalized ankle moment for all subjects.

Active				Passive			
Subject	Slope	Intercept	R^2	Subject	Slope	Intercept	R^2
1	4.78	2.64	0.72	1	14.30	3.12	0.87
2	6.70	2.20	0.97	2	14.95	2.91	0.76
3	6.87	2.94	0.88	3	17.64	0.24	0.67
4	5.73	3.38	0.97	4	24.11	2.15	0.96
5	4.46	2.15	0.66	5	5.73	2.32	0.90
6	4.04	4.38	0.97	6	23.28	2.23	0.99
7	4.77	4.26	0.83	7	25.76	2.37	0.96
8	5.98	2.62	0.97	8	11.00	3.30	0.46
9	4.12	1.96	0.77	9	17.34	2.79	0.86
10	3.04	3.22	0.98	10	1.99	2.19	0.43
Avg	5.05	2.98	0.87	Avg	15.61	2.36	0.79
St dev	1.24	0.84	0.12	St dev	7.80	0.85	0.20

between shear wave speed and active ankle moment averaged 0.9 (0.1) across the subjects tested. However, the slope of this relationship was significantly ($p = 0.002$) lower in the active case (15.6 (m s^{-1})/M/M$_{MVC}$) than in the passive case (5.1 (m s^{-1})/M/M$_{MVC}$) (table 2).

Inter-rater repeatability for the shear speed measures obtained from the images was high, with the intraclass correlation coefficient (class 2) mean value of 0.999.

4. Discussion

The objective of this study was to determine if shear wave speed would track variations in ankle plantarflexor loads induced by either passive muscle stretch or active muscle contraction. We found that shear wave speed does correlate with variations in the net mechanical loading, but the nature of that relationship varies depending on whether the muscle force was actively or passively generated.

Our passive experiments were designed to delineate the contribution of the gastrocnemius to the ankle joint moment generated. To achieve this aim, we stretched the plantarflexors with the knee in flexed and extended postures. The gastrocnemius is shortened in the flexed case such that the ankle moment primarily reflects passive loading of the soleus (Sale *et al* 1982). As expected in this case, we found that the gastrocnemius shear wave speeds did not significantly increase with dorsiflexion. However, in the extended knee posture, the gastrocnemius is more taut, such that dorsiflexion further stretches the muscle and induces a passive ankle moment at smaller dorsiflexion angles (Maisetti *et al* 2012). Correspondingly, we observed an exponential increase in shear wave speed with ankle dorsiflexion in the extended knee condition (Akagi *et al* 2012), which is consistent with prior results that have been reported (Shinohara *et al* 2010, Akagi *et al* 2012). Length dependent changes in passive muscle force are believed to arise from the stretch of titin (Horowits *et al* 1986) and connective tissues within the muscle (Gajdosik 2001). Hence, it was expected that passive stiffness would increase with muscle length, and would give rise to a monotonic increase in shear wave speed with force at stretched lengths.

We also show that muscle shear wave speed can be modulated via active muscle contraction, which would represent a neurally modulated muscle stiffening effect. Prior biomechanical studies have found a linear relationship between net ankle stiffness and the moment generated by muscles about the joint (Agarwal and Gottlieb 1977, Ochala *et al* 2004, Sinkjaer *et al* 1988). This muscle stiffening presumably arises from the increased number of activated cross-bridges (Ford *et al* 1981). This effect is consistent with prior studies that showed that muscle shear wave speed is linearly related to muscle activation level, as assessed using surface electromyography (Nordez and Hug 2010, Gennisson *et al* 2005).

A major observation of the current study is that net loading-induced changes in shear wave speed appear to be different in active and passive conditions. In particular, we observed a larger change in shear wave speed with loading under the passive conditions. This result suggests that shear wave speed cannot be used as a surrogate measure of net ankle loading in situations where the muscles are both stretched and active, which would preclude a number of functional tasks that involve substantial ankle dorsiflexion. This result could arise from a couple of factors. First, we are only able to relate shear wave speed to the net ankle moment which is due to loading of the gastrocnemius and other soft tissues (e.g. the soleus) crossing the joint. Although it is generally believed that both the gastrocnemius and soleus contribute to the induced ankle moment in an extended knee posture (Sale *et al* 1982), it is conceivable that the relative load sharing between the muscles differs between individuals and conditions. Specifically, if the gastrocnemius were to accommodate a relatively greater percentage of the ankle load in a passive condition than in the active condition, it could give rise to the steeper linear relationship between gastrocnemius shear wave speed and net ankle moment that we observed (figure 4). Unfortunately, it is not feasible to measure individual muscle loads *in vivo*, and an animal model may be necessary to investigate this factor more fully.

It is also possible that shear waves propagate differently through internal structures depending on whether the tissue is actively or passively loaded. In particular, shear wave propagation may be sensitive to anatomical features such as muscle thickness, fibre composition of tissue, the relative amount of connective tissue within the muscles and/or fascicle orientation. We performed a post-hoc analysis to assess whether variations in pennation angle or fascicle orientation with respect to the transducer may affect shear wave speed measures. To achieve this aim, we retrospectively measured the pennation angle in the *B*-mode images. We found the average change in pennation angle to be 11.1 ($\pm$ 7.0)$^\circ$ with active contraction, and 6.8 ($\pm$1.7)$^\circ$ and 6.1 ($\pm$2.3)$^\circ$ change with passive stretch in the extended and flexed postures, which is similar to the changes in pennation angle that have been reported previously (Abellaneda *et al* 2009). Perhaps more relevant to consider with respect to SWE, however, would be the change in orientation of the muscle fascicles with respect to the transducer, which would subsequently affect the angle at which the tissue is perturbed to induce shear wave speeds. We performed this analysis by measuring the fascicle angle with respect to the image plane in the data collected from passive trials. As expected, we found this angle to increase as the ankle was plantarflexed. In contrast, we found no relationship between shear wave speed and the angle measured, for either the extended knee or flexed knee cases, with the R^2 values of linear trend lines for these data of 0.0744 and 0.078 respectively.

There are some limitations to consider when interpreting the results to this study. Although shear wave speed in muscle has previously been shown to be sensitive to transducer position (Gennisson *et al* 2010), this prior study focused on measuring the effects on shear wave speed of rotating the probe in the coronal plane. In contrast, in our study, all data were collected from the sagittal plane. Likewise, prior studies have found that shear wave speed variations within muscle tissue are very low (Gennisson *et al* 2010, Nordez and Hug 2010), which is consistent with the high inter-rater repeatability that we measured for shear wave speeds. Secondly, although subjects initiated the active isometric contractions at a neutral ankle angle (0°), some ankle rotation ($<10^\circ$ at 50% MVC) did occur as they incremented up their contraction levels. Such motion can arise due to soft tissue deformation and compliance in the test device, effects which have been observed previously in similar setups (Maganaris *et al* 2002, Maganaris 2005, Muramatsu *et al* 2001). The resulting shortening of the plantarflexors could affect the shear wave speeds within the tissue, as noted during the passive stretch trials. Based on our results from the passive trials (figure 3), we would estimate that this change in angle would lead to a shear wave speed effect of less than 1 m s^{-1} on average, which would still result in a lower speed-force slope than observed in the passive loading case (figure 4).

In conclusion, our results show that SWE is a promising approach for indirectly assessing muscle loading by tracking changes in shear wave speed. However, we did observe a differential effect of active and passive loading on shear wave speed which makes it important to carefully consider the relevant loading conditions when using SWE for both clinical and research studies.

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